Summary and Conclusions
The major objectives of this study were to investigate the phytochemicals from bitter cumin seeds (*Centratherum anthelminticum* (L.) Kuntze) and their biological properties in terms of antioxidant, antidiabetic and antimicrobial activities. The major findings of this study can be summarized as follows:

1. Bitter cumin (*Centratherum anthelminticum* (L.) Kuntze or Kalijeera in vernacular name) belongs to family Asteraceae. The seeds have a sharp bitter taste.

2. Bitter cumin is used in traditional medicine for the treatment of fever, cough, and diarrhoea and also used as herbal tonic and antidiabetic agent. The seeds are reported to possess acrid, febrifugal, alterative, astringent, antihelmenthic, antulcer, antiphlegmatic, cardiac, diuretic and digestive properties.

3. Proximate analysis showed that bitter cumin seeds contained 14.7% carbohydrates, 21.4% fat, 22.5% protein, 29.3% fiber, 7.2% ash and 4.9% moisture.

4. Bitter cumin oil contained 50% linoleic acid which is one of the essential fatty acids. The other fatty acids present in bitter cumin oil are palmitic acid, palmitoleic acid, stearic acid, oleic acid and linolenic acid.

5. The total phenols of bitter cumin seeds were extracted with water, aqueous methanol and aqueous methanol acetone. Among these extracts, aqueous methanol acetone extract of bitter cumin (AMAEBC) showed highest phenolic content.

6. The AMAEBC contained a mixture of phenolic compounds such as gallic acid, protocatechuic acid, caffeic acid, ellagic acid, ferulic acid and flavonoids viz., quercetin and kaempferol as determined by LC-MS.

7. The major phenolic compound present in AMAEBC was caffeic acid. The various phenolic compounds present in bitter cumin extract was in the decreasing order of caffeic acid> ferulic acid> gallic acid> quercetin> ellagic acid> protocatechuic acid> kaempferol.
8. The biological properties of bitter cumin extract (AMAEBC) were studied on antioxidant, antidiabetic and antimicrobial systems.

9. The effect of bitter cumin extract was studied on different antioxidant systems such as DPPH, ABTS and superoxide anion radical scavenging, phosphomolybdenum and potassium ferricyanide reducing power, soybean lipoxygenase dependent lipid peroxidation, rat liver microsomal lipid peroxidation, liposomal oxidation and oxidative DNA damage.

10. Bitter cumin showed a potent antioxidant activity in the above assay systems. Among various extracts of bitter cumin, aqueous methanol-acetone extract showed highest antioxidant potential. There was a very strong correlation between total phenol content and antioxidant activity. The polyphenolic compounds present in AMAEBC were established antioxidant molecules. Therefore antioxidant activity can be attributed to the polyphenolic compounds present in bitter cumin seeds.

11. Bitter cumin extract (AMAEBC) showed significant antioxidant activity by scavenging DPPH radical, ABTS radical, superoxide anion radical, reduced phosphomolybdenum and potassium ferricyanide, inhibited lipoxygenase dependent lipid peroxidation, Fe$^{2+}$ and ascorbate induced rat liver microsomal lipid peroxidation and protected against hydroxyl radical mediated oxidative damage to DNA. Thus AMAEBC was able to scavenge or neutralize DPPH$^•$, ABTS$^{•+}$, superoxide anion radical, lipid peroxy radical, hydroxyl radical and reduce phosphomolybdenum and ferricyanide molecules.

12. The antidiabetic potential of AMAEBC was studied both in *in vitro* and *in vivo* model systems. In *in vitro* model, the effect of AMAEBC was studied on carbohydrate hydrolysing enzymes such α-amylase, sucrase, maltase and PNP-glucoside hydrolase. In *in vivo* model studies were conducted on normal rats, streptozotocin induced diabetic rats and high fructose and low streptozotocin diabetic rats.
13. AMAEBC dose dependently inhibited rat intestinal sucrase, maltase and PNP-glucoside hydrolase. The inhibitory potential of bitter cumin was less than the therapeutic α-glucosidase inhibitor acarbose, but relatively higher than DL-catechin, a reported α-glucosidase inhibitor from tea polyphenols.

14. Sucrase inhibitory activity of bitter cumin was found to be higher than maltase inhibitory activity in contrast to most of the reported natural α-glucosidase inhibitors. This property may be due to the presence of high concentration of caffeic acid in bitter cumin which is reported to possess higher inhibitory effect on sucrase activity than maltase activity.

15. Enzyme kinetic studies on α-glucosidase inhibition showed that the $K_m$ values remain unchanged with different concentrations of AMAEBC, but the $1/V_{max}$ values increased with an increase in concentration of AMAEBC. This pattern of inhibition indicates the bitter cumin inhibited α-glucosidase activity in a non-competitive manner.

16. Studies on human salivary α-amylase showed that bitter cumin can inhibit α-amylase activity in a dose dependent manner. The efficacy of bitter cumin in inhibiting α-amylase was less when compared to acarbose. But bitter cumin extract with strong inhibitory effect on α-glucosidase and weak α-amylase inhibitory activity indicates it is more ideal for the control of type 2 diabetes.

17. Bitter cumin significantly reduced postprandial hyperglycemia in overnight fasted maltose loaded normal rats confirming its potential antihyperglycemia activity. The antihyperglycemic activity was found to be better than acarbose but at a higher concentration of bitter cumin extract.

18. In streptozotocin induced diabetic rats an oral feeding of bitter cumin extract for a period of one week reduced the fasting blood glucose level compared to control streptozotocin induced diabetic rats. Bitter cumin extract with very rich phenolic content may be having insulin like activity...
or as a strong antioxidant it may be involved in the rejuvenation of β-cells, or may increase the output of insulin from the existing β-cells.

19. High fructose and low streptozotocin type 2 diabetic animal model for oral feeding of bitter cumin extract (AMAEBC) for a period of 4 weeks reduced fasting blood glucose level in a dose dependent manner.

20. Antimicrobial study of bitter cumin extract (AMAEBC) was studied on food borne pathogenic and spoilage bacteria.

21. The preliminary screening of antibacterial activity of bitter cumin extract by agar diffusion method showed that it is more effective on Gram positive bacteria than on Gram negative bacteria.

22. Bitter cumin showed marked growth inhibition of Bacillus cereus, Bacillus subtilis and Staphylococcus aureus and moderate inhibition of Listeria monocytogenes and Enterobacter spp. The species like Escherichia coli and Yersinia enterocolitica are insensitive to bitter cumin extract.

23. Minimum inhibitory concentration (MIC) of AMAEBC against Bacillus cereus, Staphylococcus aureus and Listeria monocytogenes was 50 ± 7, 260 ±18 and 700 ± 42 µg/mL respectively.

24. Scanning electron microscopy on B. cereus and S. aureus showed that bitter cumin seed extract caused changes in cell wall configurations leading to cell wall lyses and to cell death.

Future Research

This study has demonstrated that bitter cumin seeds are a rich source of an array of dietary phenolic compounds and flavonoids. The bitter cumin seeds extract showed potent antioxidant, antihyperglycemic and antibacterial activity. The mechanism of antioxidant, antihyperglycemic and antibacterial activities of bitter cumin seeds has been elucidated to certain extent.

Future areas of potential study include-
i) Further clinical investigations are required to confirm antihyperglycemic effect of bitter cumin in treatment of Type 2 diabetes.

ii) The mechanism of antifilarial activity of bitter cumin remains to be established.

iii) Studies on human cancer cells could be useful to examine whether bitter cumin seeds have anticancer property.

iv) Bitter cumin seed powder can be used to control filarial worm infections and also post prandial hyperglycemia. However toxicological and safety profiles of bitter cumin seeds need to be established.